Interactions of unsaturated fat or coconut oil with Rumensin® on milk fat production might be mediated through inhibition of specific protozoal genera.

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INTRODUCTION

Meat and milk from ruminants are rich in saturated fat and this influences human health. The saturation of fat occurs in the rumen where microorganisms hydrogenate unsaturated fatty acids (**FA**). The metabolism of the FA in the rumen also influences the synthesis of FA in the mammary gland and can be responsible for a decrease in milk fat production. The purpose of this research is to identify the ruminal microbial changes that are responsible for FA hydrogenation

Polyunsaturated fatty acids (**PUFA**) feeding often decreases protozoal numbers in the rumen. Animal-vegetable fat (**AV**), a by-product of the food industry, is readily available to provide PUFA in dairy diets. However, the response to AV supplementation on protozoal numbers is not consistent, possibly due to biohydrogenation (**BH**) of PUFA in the rumen (Oldick and Firkins, 2000). Long chain saturated FA are less toxic to protozoa; therefore, the BH of PUFA removes their potential inhibitory effects. In contrast, evidence from OSU supports the contention that protozoa are a vehicle for passage of PUFA or other intermediates of BH that do not promote MFD (Karnati et al., 2006).

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AV supplementation in combination with Rumensin® (**R**), an ionophore improving feed efficiency, occasionally spontaneously decreases milk fat yield and percentage (Duffield et al., 2003). This milk fat depression (**MFD**) is likely due to the partial BH of PUFA, which favors FA intermediates that are inhibitory to milk fat synthesis.

Feeding coconut oil (**CO**) rich in medium chain fatty acids (**MCFA**), and therefore low in PUFA, has decreased the abundance of ruminal protozoa in sheep (Machmuller et al., 2003). We hypothesized that, while lowering protozoal populations, diets supplemented with CO in combination with R would not cause MFD as would AV diets combined with R. PUFA or MCFA in combination with R could shift ruminal fermentation and potentially depress fiber degradation, reducing fed intake.

Therefore our objectives were to determine the effects of feeding AV or CO in combination with R on protozoal abundance, ruminal fermentation, total tract digestibility, feed intake, milk and milk fat production. This interaction between R and fat source on MFD is reported here with a 2x3 factorial arrangement of treatments with +/- R and either no fat, AV, or CO.

MATERIALS AND METHODS

Six primiparous rumen-cannulated Holstein cows (79 DIM) were fed six diets in a 6x6 Latin square design. The diets were supplemented or not with 260 mg/d of R (+/- R), and with

control (no fat added), 5% AV, or 5% CO in a 2x3 factorial arrangement. Periods were 3 wk except the 4-week initial period to allow adequate adaptation of ruminal populations to R; in subsequent periods, rumen contents were transferred to hasten adaptation. Diets were prepared once daily as a TMR and fed every 2 h.

All diets contained 16.2% alfalfa hay and 32.8% corn silage on a DM basis. The diet composition averaged 16.6% CP, 5.5% ash and 41.5% NFC, similar for all diets. Diets averaged 2.4, 5.8, and 6.4% of FA for control, AV, and CO, respectively, and the FA profile is reported in **Fig. 1**. The diets had 33.6, 29.6, and 28.5 %NDF for control, AV, and CO, respectively. The R was measured and verified within expected ranges in the 3 supplemented diets.

Measurement of protozoal counts were performed as described in Dehority and Odenyo (2003); ruminal fermentation analysis, total tract digestibility and lactation performances measurement were performed as described previsously (Reveneau et al., 2005).

The mixed model included fixed (diet) and random (period, cow) effects. Contrasts were the main effects of: 1) Rum (+/- R), 2) Fat supplementation (control vs. AV+CO), and 3) Source of fat (AV vs. CO); and 4 and 5) the interactions of R with contrasts 2 (RxF) and 3 (RxS). Significance was $P<0.05$ for main effects and $P<0.10$ for interactions.

RESULTS AND DISCUSSION

Total protozoal counts (cell/ml rumen fluid) were decreased by one log with the addition of CO in the diet (**Table 1**.). More specifically, Isotricha decreased by two log and Entodinium by one log with CO, whereas Epidinium number were maintained (**Fig.2**). With the addition of R, Epidinium decreased by 67% and when AV was supplemented with R, the decline was more severe (92%).

Total VFA concentration (130 mM) was not changed by diet (**Table 2.**). The molar percent of acetate was decreased by fat and fat source. In contrast, propionate molar percent was increased. The addition of CO had the greatest impact on the acetate to propionate ratio, with 2.95, 2.58, and 1.85 for control, AV, and CO respectively. In accordance with the decrease in protozoal numbers, butyrate molar percent was decreased with CO addition compared to AV. Total tract apparent digestibility of OM was not different with diet (**Table 3**). Fat supplementation decreased total tract NDF digestibility. Apparent total tract digestibility of C18 and total fatty acids was higher with CO. R supplementation increased apparent total tract digestibility of total C18 fatty acid. CO supplementation lowered DMI by 5 kg/d and milk production was also decreased with Rand with CO supplementation (**Table 4.**). The decrease in milk yield characteristic of MFD occurred with AV+R and CO.

Feeding CO drastically decreased protozoal cell counts and shifted ruminal fermentation toward propionate at the expense of acetate and butyrate. Although total protozoal counts were not affected by the interaction RxS, the counts of *Epidinium* were lower when fed AV+R.

Because this diet also caused MFD, *Epidinium* may be involved in BH mechanisms. Recent data supports the involvement *Epidinium* in the flow of BH intermediates to the duodenum (Devillard et al., 2006).

Against our hypothesis, diets supplemented with CO also induced MFD, possibly through another mechanism than AV diets. Analyzing the milk fat composition will clear the role of trans FA in the response that has been observed in research with lauric acid in the past (Dohme et al., 2004). Since feeding CO differentially affected protozoal genera with no toxic effects on *Epidinium,* more research is needed to clearly identify the mechanisms of this resistance

The changes in VFA were associated with a decreased total tract digestibility of NDF for CO from inhibition of fiber degradation in the rumen. This inhibition is associated by the lower DMI with CO from rumen fill. Total tract digestibly of FA was higher with CO due to higher duodenal digestibility of MCFA. Higher C18 digestibility with R could result from more UFA from incomplete BH, causing the MFD observed when AV+R was fed (Griinari and Bauman, 2001).

Further analyses should elucidate the role of protozoal concentration and genera on bacterial biohydrogenation in the rumen. Although the inhibition of fiber degradation might have limited energy for milk fat synthesis, further analysis of omasal and milk FA will help elucidate the mechanism of MFD with CO supplementation.

CONCLUSIONS

The implication of this research is that although medium chain FA are not a good alternative to prevent MFD in dairy cows, their use can help identify the ruminal changes promoting MFD. *Epidinium* displayed resistance to MCFA toxicity and may be involved in BH mechanisms. As a result, we will better understand the mechanism of saturation of FA in the rumen responsible for high-saturated FA in meat and milk and possible modification of this mechanism.

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Figure 1. FA profile in % of total FA in control, AV and CO diets.

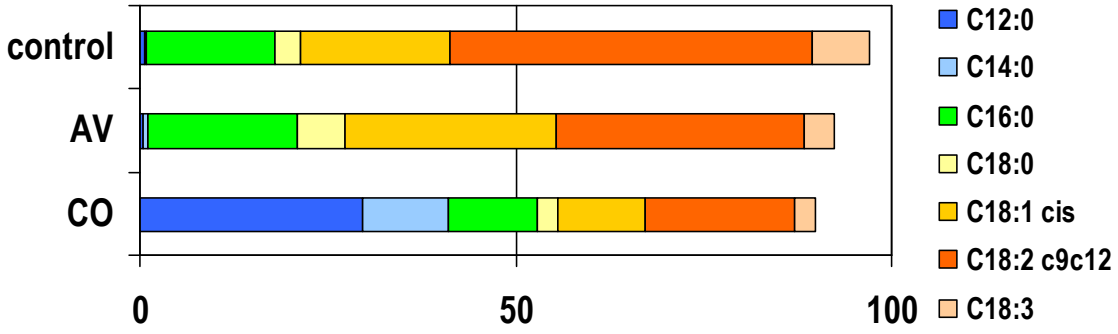

Figure 2. Panel of micrographs of protozoan cells from rumen contents of cows on AV or CO diets.
A. *Ophryoscolex* using phase contrast (400X)
B. Mixed protozoa cells stained with methyl blue and Lugol's reagent (100X)
C. *Epidinium* using phase contrast (400X)

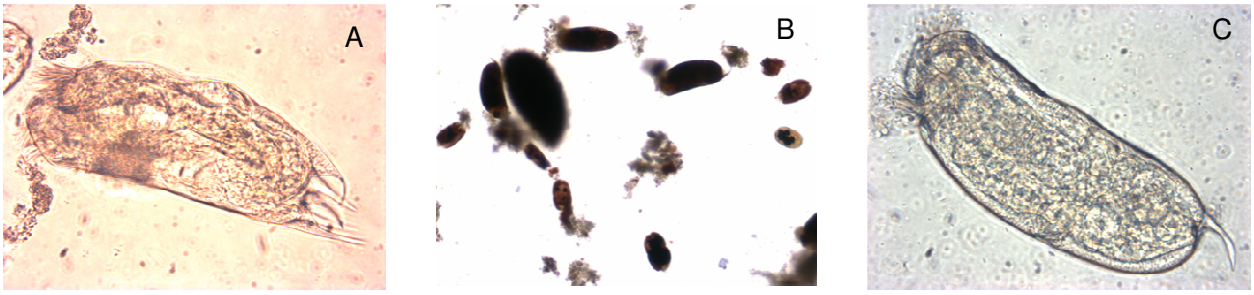

Table 1. LS means of log 10 for total and genera specific protozoan cell counts per ml rumen fluid.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
Total	5.91	5.86	4.86	6.01	5.98	4.75	0.14	NS	<0.01	<0.01	NS	NS
Isotricha	3.89	4.25	2.24	3.90	3.99	1.82	0.40	NS	<0.01	<0.01	NS	NS
Dasytricha	3.30	2.41	0.74	3.81	3.32	1.29	0.50	0.07	<0.01	<0.01	NS	NS
Entodinium	5.85	5.79	4.65	5.96	5.93	4.44	0.17	NS	<0.01	<0.01	NS	NS
Epidinium	2.97	3.73	3.42	2.43	1.88	3.22	0.83	<0.01	NS	0.13	NS	0.02

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

Table 2. LS means for ruminal fermentation.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
Total VFA, mM	133	125	129	144	127	123	5	NS	<0.01	NS	0.12	NS
VFA, mol/100 mol												
Acetate	62.2	60.0	54.7	62.7	57.7	54.4	1.0	NS	<0.01	<0.01	0.13	0.16
Propionate	21.5	23.4	30.2	21.5	24.5	30.5	1.6	NS	<0.01	<0.01	NS	NS
Butyrate	12.4	12.7	10.5	11.7	13.6	11.0	1.0	NS	NS	<0.01	0.17	NS

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

Table 3. LS means of apparent nutrient digestibility of the total tract.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
OM	68.2	68.0	67.7	65.7	70.7	67.3	1.5	NS	NS	NS	0.17	NS
NDF	56.7	46.0	30.5	49.8	47.0	40.5	3.7	NS	<0.01	<0.01	0.04	0.19
FA	69.2	69.5	88.2	72.8	74.7	91.0	4.4	0.16	<0.01	<0.01	NS	NS
Total C18	73.5	68.5	81.0	76.8	75.0	88.7	4.5	0.05	NS	<0.01	NS	NS

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

Table 4. LS means of dry matter intake and milk production.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
DMI, kg/d	20.0	19.8	15.5	19.3	19.0	14.8	0.7	0.08	<0.01	<0.01	NS	NS
Milk, kg/d	33.9	34.3	30.5	33.1	31.7	30.1	2.0	0.06	<0.01	<0.01	NS	0.16
Milk fat, g/d	1.08	1.01	0.71	1.05	0.87	0.74	0.05	0.15	<0.01	<0.01	NS	0.08

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).